Declaration

I, Koji HIRAYAMA, Patent Attorney, residing at c/o Nakamura & Partners, 3-1, Marunouchi 3-chome, Chiyoda-ku, Tokyo, JAPAN, do hereby certify that I am conversant with the English and Japanese languages and am a competent translator, thereof, and that to the best of my knowledge and belief the attached English translation is a true and correct translation made by me of U. S. Serial No. 10/767,436 entitled NOVEL DITERPENE COMPOUNDS.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 25th day of May, 2004

K. Hirayama

Koji HIRAYAMA
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NOVEL DITERPENE COMPOUNDS

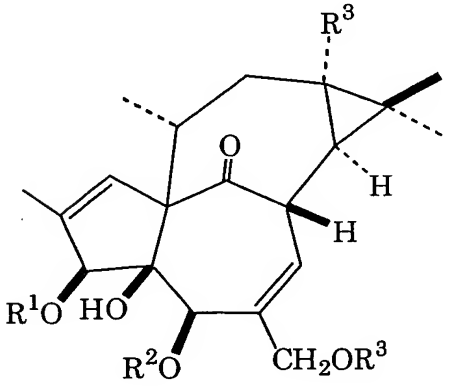
(Technical field of the invention)

The present invention relates to novel diterpene compounds isolated from Kan Sui, which is useful as antineoplastics.

(Prior art)

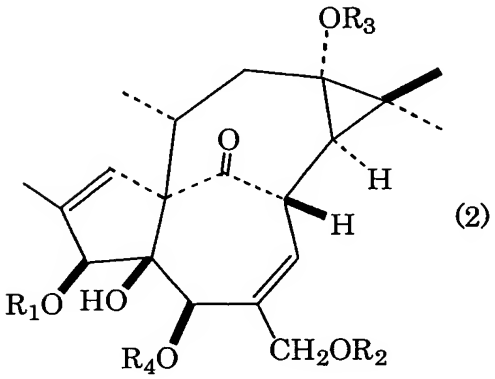
Kan Sui (Euphorbia kansui L.) is a perennial plant belonging to Euphorbiaceae and growing in northwest China. Kan Sui has been used in China as herbal remedy for treating allergic diseases such as chronic bronchitis and bronchial asthma, and malignant tumors such as esophagus cancer and breast cancer. Investigations of components in Kan Sui began in about 1943 and over ten diterpene and triterpene compounds have been found to date. Recent studies have shown that some of these diterpene compounds have anticancer activity, antiviral activity and cytotoxic activity, and thus, many investigators pay attention to these compounds.

S. M. Kupchan et. al. showed that Ingenol derivatives extracted from Euphorbia esula L. and represented by the general formula (1) below have depressive activities against P-388 lymphocytic leukemia in mice. (S. Morris Kupchan, et. al., Science, Vol. 191, pp. 571-572, 13, Feb., 1976.)

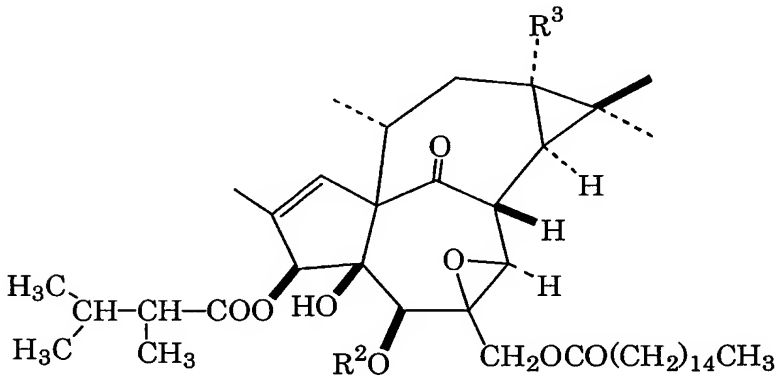


(1)

Tian-Shung Wu et. al. showed that kansuiphorin A and kanusiphorin B extracted from the roots of Euphorbia kansui L. and represented by the general formulae (2) and (3) respectively, have depressive activities against P-388 cells in vivo. (Tian-Shung Wu, et. al., J. Nat. Prod., Vol. 54, No. 3, pp. 823-829, 1991.)

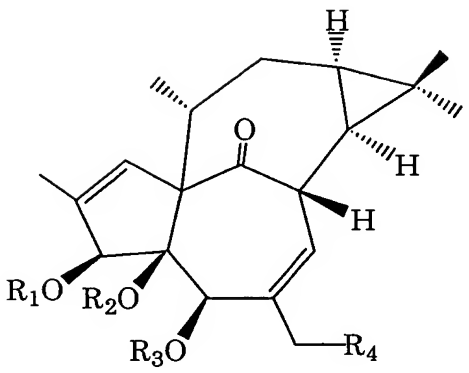


(2)



(3)

Magdalena Blanco-Molina et. al. stated that Ingenol derivatives represented by the general formula (4) below were possible to induce apoptosis in Jurkat cells. (Magdalena Blanco-Molina, et. al., Chemistry & Biology, 8/8, pp. 767-778, 2001.)



(4)

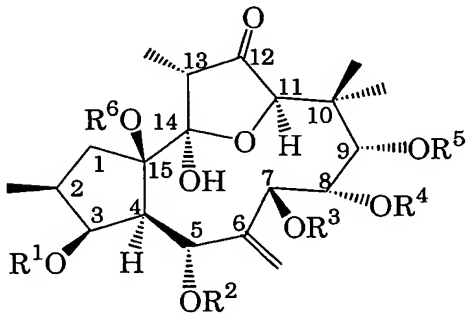
However, any prior art does not disclose compounds of the present invention.

(Problem to be solved)

An object of the present invention is to provide novel diterpene compounds with antineoplastic activities.

(Means for solving the problem)

Novel diterpene compounds of the present invention are represented by the following general formula (I).



(I)

in which,

R^1, R^2, R^3, R^4, R^5 and R^6 may be same or different and represent hydrogen atoms, liner or branched, saturated or unsaturated, substituted or unsubstituted aliphatic groups, or radicals represented by the general formula RCO-, wherein R

denotes a liner or branched, saturated or unsaturated, substituted or unsubstituted aliphatic group, a substituted or unsubstituted aromatic or heteroaromatic group.

The compounds represented by the formula (I) are useful as a therapeutic agent for treating malignant tumors, such as esophagus cancer, breast cancer, and so on.

(Mode for carrying out the invention)

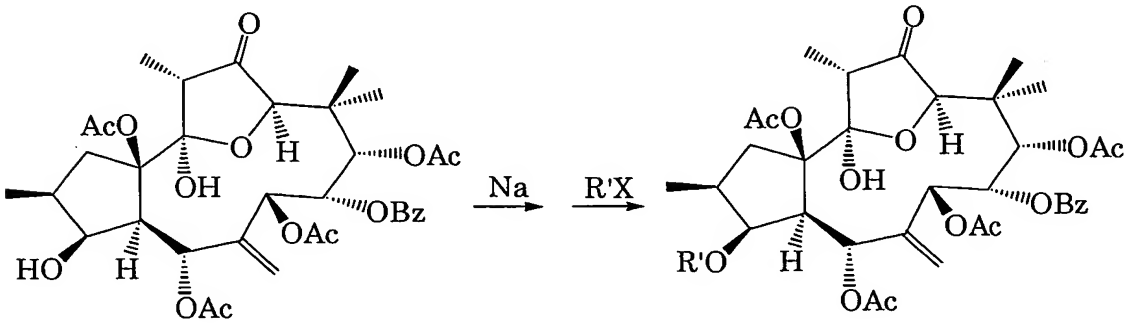
R^1, R^2, R^3, R^4, R^5 and R^6 in the formula (I) represent preferably liner or branched, saturated or unsaturated aliphatic groups containing preferably from 1 to 30 carbon atoms, and more preferably from 1 to 16 carbon atoms. The aliphatic group may be substituted with a halogen atom, a hydroxyl group, an ether group, a carbonyl group, a carboxyl group, an amino group and an amide group.

Examples of carboxylic acids from which the RCO· radical wherein R represents an alkyl radical is derived include saturated aliphatic acids containing from 1 to 16 carbon atoms, such as acetic acid, propionic acid, butyric acid, 2,3·dimethyl butanoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, and unsaturated aliphatic acids containing from 3 to 16 carbon atoms, such as 2,4·decadienoic acid. Examples of carboxylic acids from which the RCO· radical wherein R represents an aromatic radical is derived include aromatic carboxylic acids, such as benzoic acid, phthalic acid, salicylic acid, and anthranilic acid. Examples of caboxylic acid from which the RCO· radical wherein R represents an heteroaromatic radical is derived include furancarboxylic acid, thiophenecarboxylic acid, pyridinecarboxylic acid, such as nicotinic acid and iso·nicotinic acid. The aromatic and heteroaromatic radicals may be substituted with halogen atoms, hydroxyl radicals, ether radicals, carbonyl radicals, carboxyl

radicals, amino radicals, and amide radicals.

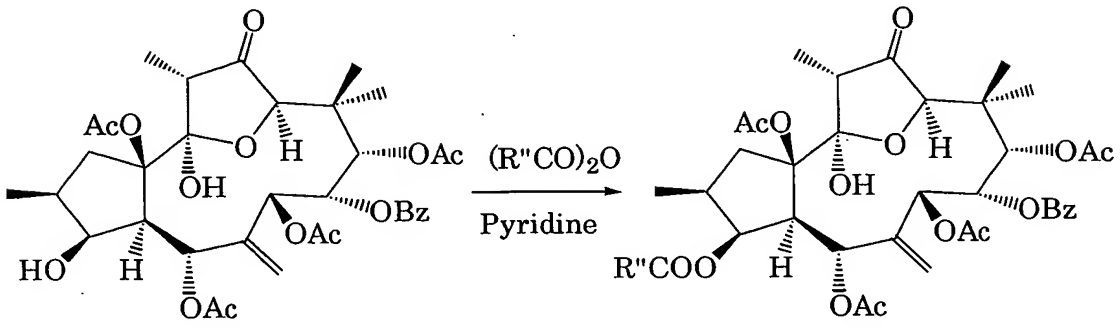
A compound represented by the general formula (I), wherein R^1 to R^3, R^5 and R^6 are acetyl radicals and R^4 is a benzoyl radical (Compound 5) and a compound represented by the general formula (I), wherein R^1 to R^3 and R^6 are acetyl radicals, R^4 is a benzoyl radical and R^5 is a nicotinoyl radical (Compound 8) are prepared by extracting raw or dried, and preferably ground roots of Euphorbia kansui L. with organic solvents, such as chloroform, ethyl acetate and butanol at a room temperature, and then purifying the extracts according to known procedures.

These extracted compounds may be used as starting materials for the preparation of other compounds represented by the general formula (I). For example, Compound 5 is hydrolyzed to give the compound of formula (I) wherein R^1 is a hydrogen atom, and then the obtained hydroxyl compound is converted to ether compounds of the present invention in accordance with the Williamson Synthesis described below, that is, by the action of sodium alkoxides and then alkyl halides R'X wherein R' has the same meaning as R^1 and X represents a halogen atom:



Further, the hydrolyzed Compound 5 wherein R^1 is a hydrogen atom may be reacted with acid anhydrides, i. e., $(R"CO)_2O$ as described below in the presence of

anhydrous pyridine to give ester compounds of the present invention.



Example 1

To 15 Kg of the dried roots of Euphorbia kansui L. collected in People's Republic of China were ground and extracted with 45 L of 60 % aqueous ethanol (v/v) for 12 hours at a room temperature with stirring. The extraction was repeated again under the same conditions. The extracts were combined together and concentrated under reduced pressure at 40 ℃ to give a concentrated extract (1200 g). The extract was dissolved in 4 L of water and extracted with chloroform (3 x 4 L), ethyl acetate (3 x 4 L) and n-butanol (3 x 4 L) in this order. These fractions were concentrated under reduced pressure to give 165 g, 23 g and 64 g of concentrated extracts from the chloroform, ethyl acetate and n-butanol fractions, respectively.

150 g of the extract obtained from the chloroform fraction was subjected to silica gel chromatography (Wako gel C-300, Wako Pure Chemical Industry, 13 x 22 cm) and eluted with hexane/ethyl acetate gradually changed from whose ethyl acetate concentration is 0 %, 2 %, 3 %, 5 %, 10 %, 20 %, 30 %, 50 %, to 100 % to give fraction Nos. 1 to 9.

Example 2

The fraction No. 6 obtained in Example 1 (a fraction eluted with a hexane solution containing 20 % of ethyl acetate) was applied to reversed phase column

chromatography (ODS-7515-12A, SSC) and eluted with water/methanol gradually changed from whose water concentration is 70 %, 50 %, 40 %, 30 %, 10 %, to 0 % to give fraction Nos. 1 to 6.

The fraction No. 4 (a fraction eluted with a methanol solution containing 30 % water) was purified by normal phase HPLC. Silica gel (Shenshu- PEGASIL SILICA-60-5, 250 x 10 mm) was used as a stationary phase and chloroform : hexane : ethyl acetate = 20 : 65 : 15 (v/v/v) was used as a mobile phase. The fraction was eluted at a flow rate of 4 ml/min (room temperature) and eluents were monitored by a UV detector at 254 nm and eluents having retention times of 15.95 min and 17.43 min were fractionated respectively. These two eluents were concentrated under reduced pressure to give Compound 3 (yield: 50.1 mg) and Compound 4 (yield: 16.1 mg) as colorless waxy materials.

Example 3

The No. 6 fraction obtained in Example 1 (a fraction eluted with a hexane solution containing 20 % of ethyl acetate) was applied to reversed phase column chromatography (ODS-7515-12A, obtained from SSC) and eluted with water/methanol gradually changed from whose water concentration is 70 %, 50 %, 40 %, 30 %, to 10 % to give fraction Nos. 1 to 5. The fraction No.5 (a fraction eluted with a methanol solution containing 10 % of water) was purified by normal phase HPLC. A reverse phase column (Shenshu- PEGASIL, obtained from ODS, 250 x 10 mm) was used and acetonitrile : water = 10 : 1 (v/v) was used as a mobile phase. The fraction was eluted at a flow rate of 4 ml/min (room temperature) and eluents were monitored by a RI detector (shodex RI-101) and eluents having retention times of 31.1 min and 37.2 min were fractionated respectively. These two eluents were concentrated under reduced pressure to give Compound 1 (yield: 12.1 mg) and Compound 2 (yield: 10.1 mg) as colorless waxy materials.

The fraction No. 4 (a fraction eluted with a methanol solution containing 30 % of water) was purified by a normal phase HPLC. Silica gel (Shenshu· PEGASIL SILICA·60·5, 250 x 10 mm) was used as a stationary phase and chloroform : hexane : ethyl acetate = 30 : 10 : 10 (v/v/v) was used as a mobile phase. The fraction was eluted at a flow rate of 4 ml/min (room temperature) and eluents were monitored by a UV detector at 254 nm and eluents having retention times of 8.5 min and 9.18 min were fractionated respectively. These two eluents were concentrated under reduced pressure to give Compound 3 (yield: 14.0 mg) and Compound 4 (yield: 1.3 mg) as colorless waxy materials.

Example 4

The fraction No. 7 obtained in Example 1 (a fraction eluted with a hexane solution containing 30 % of ethyl acetate) was applied to reversed phase column chromatography (ODS·7515·12A, obtained from SSC) and eluted with water/methanol gradually changed from whose water concentration is 70 %, 50 %, 40 %, 30 %, to 10 % to give fraction Nos. 1 to 5.

A solvent of the fraction No. 2 (a fraction eluted with a methanol solution containing 50 % of water) was evaporated to give a crystal. Recrystalization of the crystal from methanol gives Compound 5 (yield: 200 mg) as a white needle crystal.

The fraction No. 1 (a fraction eluted with a methanol solution containing 70 % of water) was purified by reversed phase HPLC. FluoFix (type: IEW205) was used as a stationary phase and 45 % acetonitrile solution (acetonitrile : water = 45 : 55 (v/v)) was used as a mobile phase. The fraction was eluted at a flow rate of 6.0 ml/min (room temperature) and eluents were monitored by a UV detector at 210 nm and eluents having retention times of 11.0 min and 12.67 min were

fractionated respectively. These two fractions were evaporated and recrystalized from methanol to give Compound 6 (yield: 20.0 mg) and Compound 7 (yield: 10.0 mg) as a white powder and a white needle crystal respectively.

The fraction No. 3 (a fraction eluted with a methanol solution containing 40 % of water) was purified by HPLC. PEGASIL ODS-2 (obtained from SSC, 250 x 10 mm) was used and 60 % acetonitrile solution (acetonitrile : water = 60 : 40 (v/v)) was used as a mobile phase. The fraction was eluted at a flow rate of 3.0 ml/min (room temperature) and eluents were monitored by a UV detector at 210 nm and an eluent having retention time of 26.05 min was fractionated. The fraction was evaporated and recrystalized from methanol to give Compound 8 (yield: 40.1 mg) as a white needle crystal.

The MS, UV and IR data for Compounds 1 to 8 in Table 1, ^{1}H NMR data for Compounds 1 to 4 in Table 2, ^{13}C NMR data for Compounds 1 to 4 in Table 3, ^{1}H NMR data and ^{13}C NMR data for compounds 5 to 7 in Table 4, ^{1}H NMR data and ^{13}C NMR data for Compound 8 in Table 5.

Table 1 MS, UV, IR data for Compounds 1 to 8

Compound	1	2	3	4
form	colorless oil	colorless oil	colorless oil	colorless oil
HR-MS (m/z)	FAB–	EI	EI	FAB+
found	685.43168	628.43401	482.30318	505.29259(M+Na)
calculated	685.43152	628.43390	482.30320	505.29300($C_{30}H_{42}O_5$+Na)
molecular weight	686	628	482	628
molecular formula	$C_{40}H_{62}O_9$	$C_{38}H_{60}O_7$	$C_{30}H_{42}O_5$	$C_{30}H_{42}O_5$
UV λ_{max}^{MeOH} nm (lg ε)	206 (2.88)	206 (2.88)	206 (4.11) 220 (3.94)	205 (4.01) 220 (3.87)
IR $\nu^{cm^{-1}}_{max}$	3440 (OH) 1742, 1725, 1718 (C=O)	3460 (OH) 1741, 1728, 1716 (C=O)	3456 (OH) 1720 (C=O)	3443 (OH) 1719 (C=O)

Table 1 (continued)

Compound	5	6	7	8
form	white needle crystal	white powder	white needle crystal	white needle crystal
HR-EI-MS(m/z)				
found	730.28401	722.25664	722.25154	793.29422
calculated	730.28361	722.25735	722.25735	793.29454
molecular weight	730	722	722	793
molecular formula	$C_{37}H_{46}O_{15}$	$C_{38}H_{43}O_{14}$	$C_{38}H_{43}O_{14}$	$C_{41}H_{47}NO_{15}$
UV λ^{nm}_{max}(lg ε)	230 (4.00)	231(4.23)	230(4.21)	225(4.10)
IR $\nu^{cm^{-1}}_{max}$	3545 (OH) 1738 (C=O) 1648 (C=C)	3504 (OH) 1712 (C=0) 1650 (C=C)	3509 (OH) 1741 (C=O) 1711 (C=O) 1651 (C=C)	3452 (OH) 1739 (C=O) 1652 (C=C)

Table 2 ^{1}H NMR data for Compounds1, 2, 3, and 4 (300 MHz, $CDCl_3$, TMS, δ (ppm) (J=Hz)

Attribution	1	2	3	4
H-1	6.01 d (1.5)	6.03 d (1.8)	6.07 d (1.5)	6.07 d (1.5)
H-3	5.44 s	5.35 s	5.53 s	5.48 s
H-5	3.88 d (6.9)	3.69 s	3.68d (6.9)	3.68 brs
H-7	6.07 d (3.9)	5.71 m	5.77 m	5.76 m
H-8	4.06 dd (12.6, 4.5)	3.98 m	4.01 dd (11.7, 3.6)	4.01 dd (11.7, 3.6)
H-11	2.56 m	2.54 m	2.46 m	2.44 m
H_2-12	2.72 dd (16.8, 3.3)	2.70 dd (16.8, 3.3)	2.26 m[b]	2.26 ddd (15.9, 9.0, 3.3)
	2.19 m[b]	2.20 m[b]	1.75 m[b]	1.75 m[b]
H-13			0.67 m	0.67 m
H-14	1.23 m[b]	1.23 m[b]	0.90 m[b]	0.90 m[b]
Me-16	1.07 s	1.05 s	1.05 s	1.05 s
Me-17	1.19 s	1.18 s	1.08 s	1.08 s
Me-18	0.97 d (7.5)	0.97 d (6.6)	0.98 d (6.9)	0.98 d (6.9)
Me-19	1.78 d (1.5)	1.773 d (1.5)	1.79 m[b]	1.79 m[b]
H-20	4.73, 4.47 Abq (12.6)	1.777 s 3H	1.79 m[b]	1.79 m[b]
3-R_1	2.31 m 1H	2.31 m 1H	2′ 5.94 d (15.3)	2′ 5.85 d (15.3)
	1.92 m 1H	1.92 m 1H	3′ 7.68 dd	3′ 7.33 m
	0.92 d (6.9) 3H	0.92 d (6.9) 3H	(15.3, 11.7)	4′ 6.19 m[b]
	0.96 d (6.6) 3H	0.96 d (6.6) 3H	4′ 6.16 dd (11.7,	5′ 6.19 m[b]
	1.14 d (7.2) 3H	1.14 d (7.2) 3H	10.5)	6′ 2.15 m
			5′ 6.14 m[b]	7′ 1.44 m
			6′ 2.33 m	8′, 9′ 1.29 m
			7′ 1.43 m	10′ 0.89 t (7.0)
			8′, 9′ 1.29 m	
			10′ 0.89 t (7.0)	
13-R_3	2.19 t (7.5) 2H	2.19 t (7.5) 2H		
	1.55 m 2H	1.55 m 2H		
	1.25 s -$(CH_2)_8$-	1.25 s -$(CH_2)_8$-		
	0.88 t (6.9) 3H	0.88 t (6.9) 3H		
20-R_2	20-$COCH_3$ 2.05 s 3H			

Table 3 ^{13}C NMR data for Compounds 1, 2, 3 and 4 (75 MHz, $CDCl_3$, TMS)

C	1	2	3	4
1	131.1	131.6	132.8	132.8
2	135.8[b]	135.3	135.8	135.8
3	82.4	82.8	83.4	83.4
4	84.3	84.4	85.2	85.2
5	74.6	76.2	77.6	77.6
6	135.9[b]	137.2	137.6	137.6
7	127.9	122.7	124.3	124.3
8	42.6	42.5	43.6	43.6
9	204.6	205.1	207.1	207.1
10	71.7	71.6	72.2	72.3
11	37.5	37.8	39.1	39.1
12	35.0	34.9	31.4	31.4
13	68.8	68.8	23.3	23.3
14	28.2	28.4	23.5	23.5
15	30.2	30.3	24.2	24.2
16	22.4	22.4	28.7	28.8
17	16.6	16.7	15.7	15.7
18	18.2	18.0	17.4	17.4
19	15.5	15.5	15.8	15.8
20	66.3	21.9	22.2	22.2
3-R^1				
1′	176.7	176.9	168.0	168.1
2′	46.3	46.3	120.1	118.2
3′	31.0	31.0	141.5	146.6
4′	20.6	20.6	126.4	128.4
5′	19.1	19.1	145.4	147.1
6′	14.06	14.06	31.6	33.2
7′			28.5	28.5
8′			29.2	31.6
9′			22.5	22.6
10′			14.2	14.2
20-R^2				
1″	170.4			
2″	21.0			
13-R^3				
1‴	173.4	173.5		
2‴	34.3	34.3		
3‴	24.7	24.7		
4‴	29.48	29.48		
5‴	29.48	29.48		
6‴	29.34	29.34		
7‴	29.22	29.22		
8‴	29.15	29.15		
9‴	29.10	29.11		
10‴	31.79	31.79		
11‴	22.60	22.60		
12‴	13.98	13.97		

[b] Assignments may be interchanged.

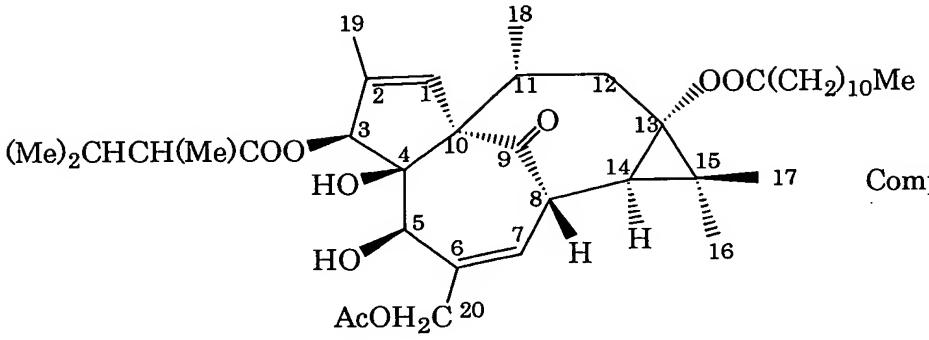
Table 4 ^{1}H, ^{13}C NMR data for Compounds 5 – 7 [(500 MHz and 125 MHz, $CDCl_3$, TMS, δ (ppm) (J = Hz))

	7		6		5	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	4.93 s	83.4	4.32 d (3.9)	87.1	2.65 dd (6.4,13.9) 2.20 m	40.3
2		80.0		78.3	2.12 m	38.8
3	5.38 d (4.9)	79.7	5.54 d (4.9)	76.7	5.58 m	74.4
4	3.48 m	46.4	3.61 dd (11.3, 4.8)	45.1	2.97 brs	51.4
5	5.95 s	74.3	5.91 m	73.8	6.13 s	70.1
6		135.8		135.8		145.4
7	5.87 s	65.1	5.89 m	64.6	6.39 s	69.1
8	4.70 d (9.4)	72.5	4.65 d (9.2)	72.7	6.05 s	71.0
9		209.3		209.5	5.07 s	82.4
10		47.9		48.1		41.5
11	3.65 d (2.1)	61.2	3.69 d (2.2)	60.8	4.13 s	77.5
12	3.43 m	58.0	3.33 dd (2.4, 9.4)	59.0		214.0
13	3.26 m	41.6	3.93 m	42.6	2.28 q (6.5)	50.7
14		211.4		204.9		106.3
15		84.8		96.1		90.6
16	1.32 s	19.4	1.31 s	20.2	0.92 d (6.3)	13.3
17	6.31 s 5.91 s	127.9	6.52 brs 5.94 brs	128.3	5.24 s 5.14 s	106.3
18	1.33 s	21.6	1.34 s	21.6	1.29 s	18.6
19	0.85 s	18.9	0.85 s	19.0	1.14 s	22.1
20	1.66 d (6.4)	19.2	1.52 d (6.4)	17.0	1.30 d (6.5)	9.21
Acetyls			Acetyls		Acetyls	
CO-1		170.6	CO-15	172.4	CO-3, 15	169.5 170.2
COMe-1	2.13 s	20.3	COMe-15 2.31 s	21.3	COMe-3,15 1.98 s 2.09 s	22.0 21.3
CO-3		169.8	CO-3	168.8	CO-5	168.8
COMe-3	1.95 s	20.6	COMe-3 1.89 s	20.4	COMe-5 1.91 s	20.9
					CO-7	170.3
					COMe-7 2.18 s	21.1
					CO-9	169.2
					COMe-9 2.07 s	20.4
Benzoyls			Benzoyls		Benzoyls	
CO-5		164.8		164.9	CO-8	165.4
COPh-5 1		128.3		128.3	COPh-8 1	130.1
2,6	7.48 m	129.1	7.55 m	129.3	2,6 8.03 m	129.9
3,5	6.88 m	127.7	7.02 m	127.7	3,5 7.42 m	128.3
4	7.09 m	132.6	7.22 m	132.8	4 7.53 m	132.9
CO-7		166.2		166.0		
COPh-7 1		128.6		128.4		
2,6	7.53 m	129.4	7.50 m	129.5		
3,5	7.02 m	127.9	6.92 m	127.8		
4	7.24 m	132.9	7.11 m	132.8		
OH-2	2.32 s		OH-2 2.52 s			
OH-8	3.53 d (9.4)		OH-8 3.54 d (9.4)			
OH-15	4.11 s		OH-1 3.92 d (3.9)			

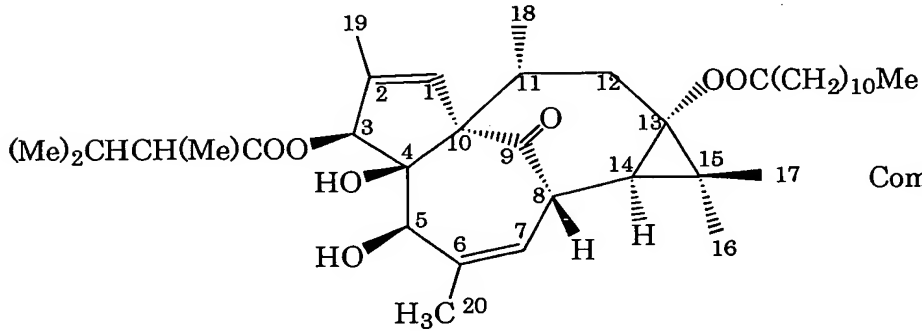
Table 5 NMR Spectral Data of 8 (300 MHz and 75 MHz)

	8	
atom	^1H	^{13}C
1 α	2.68 dd (4.5, 12.0)	39.9
1 β	2.19 m[b]	
2	2.20 m[b]	38.7
3	5.58 brs	74.1
4	3.05 d (2.7)	51.4
5	6.19 s	69.5
6		144.7
7	6.43 s	69.1
8	6.17 s	71.0
9	5.36 s	83.3
10		41.7
11	4.30 s	77.4
12		213.6
13	2.33 q (6.6)	50.9
14		106.4
15		90.5
16	0.92 d (6.0)	13.2
17a	5.17 s	109.9
17b	4.99 s	
18	1.21 s	22.1
19	1.40 s	18.7
20	1.32 d (6.5)	9.18
3-COMe	2.09 s	169.5
		21.2
5-COMe	1.95 s	168.3
		20.8
7-COMe	1.51 s	170.0
		20.3
15-COMe	2.00 s	168.8
		21.9
8-benzoyl		164.9
		129.7
	8.03 m	129.5
	7.41 m	127.8
	7.55 m	133.1
9-nicotinoyl	9.18 s	162.7
	8.77 d (4.2)	152.7
	8.31 d (7.8)	150.0
	7.39 m[b]	137.4
		125.0
		123.2

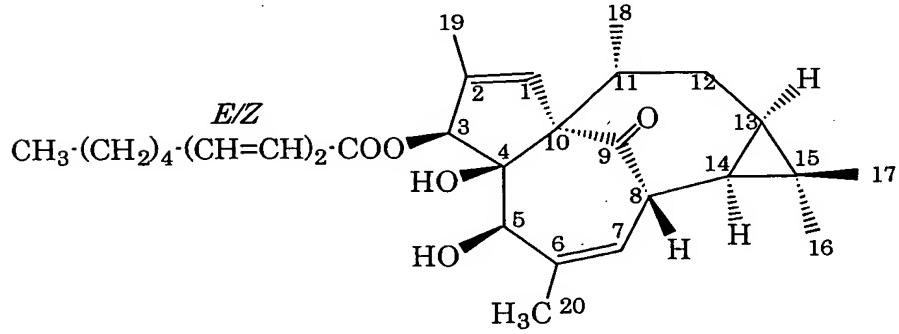
Accordingly, the structures of Compounds 1 to 8 were identified from the data above as follows.



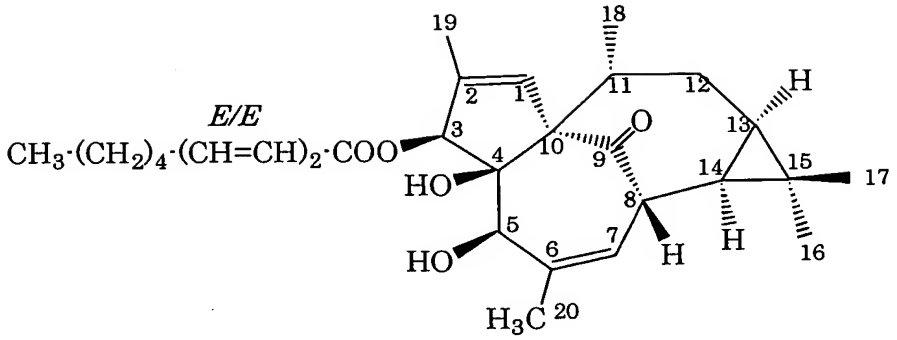
Compound 1



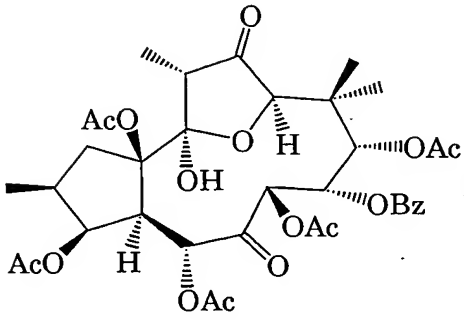
Compound 2



Compound 3

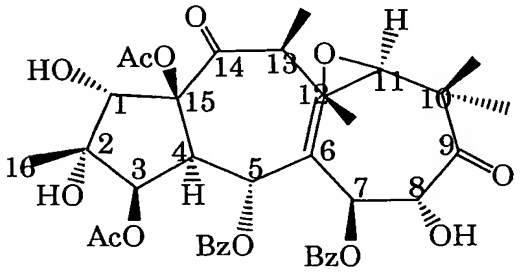


Compound 4



Compound 5

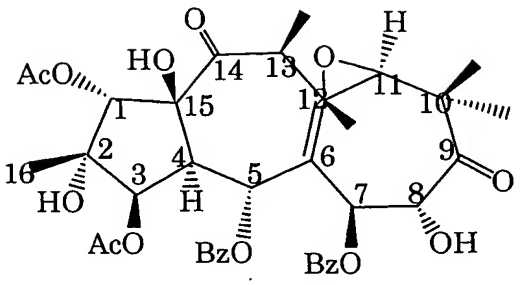
wherein, Ac represents an acetyl radical and Bz represents a benzoyl radical.



Copound 6

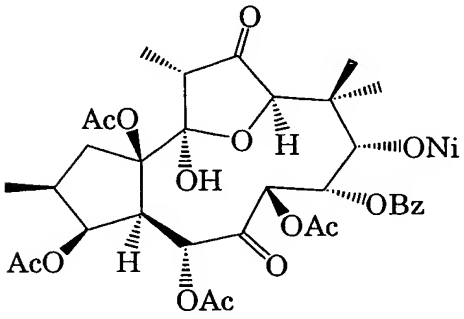
5

wherein, Ac represents an acetyl radical and Bz represents a benzoyl radical.



Compound 7

wherein, Ac represents an acetyl radical and Bz represents a benzoyl radical.



Compound 8

wherein, Ac represents an acetyl radical, Bz represents a benzoyl radical and Ni represents a nicotinoyl radical.

The compounds of the present invention have an inhibitory activity of cell growth as determined by an animal cap assay described in detail by S. F. Godsave and J. M. W. Slack, in Dev. Biol. 1989, 134: 486-490.

Animal cap cells were dissected from Xenopus laevis at the later blastular stage. Single cells were separated from the animal cap cells and transferred to medium to prepare a cell dispersion solution.

The cell dispersion solution was added to a well of a Terasaki plate filled with a 50 % animal medium containing 0.2 mg/ml of gamma-globulin. A solution containing 10 microgram/ml of the compound according to the present invention is added to the well and cultured. Next day, cell division was observed under a microscope. The inhibitory action of the compound tested is expressed a ratio of

the number of non-divided cells to the total number of the cells. The results are shown in Table 6.

Table 6 Inhibitory activity of cell division

Compound No.	Inhibitory ratio (%)
Compound 3	53.0 %
Compound 4	55.1 %

The concentration of a compound is 10 microgram/ml.

Table 6 shows apparently that the compounds of the present invention have an inhibitory activity to cell division, and therefore, they can be useful as a therapeutic agent for treating malignant tumor, such as esophagus cancer, breast cancer, etc.

The active compounds of the present invention may be administered orally, parenterally or subcutaneously. Generally doses may be preferably from 0.1 mg/day/kg to 100 mg/day/kg for adult.

The compounds of the present invention may be administered in various forms, such as tablets, powders, granules, capsules, injections, suppositories, ointments, and cataplasms. The pharmaceutical composition containing the active compounds of the present invention may be formulated by using conventional carriers and additives such as vehicles such as resolvents, bases, diluents, fillers, adjuvants such as solution adjuvants, emulsifying agents, dispersers, disintegrants, solubilizers, viscosity-increasing agents, and lubricants, and additives such as antioxidants, preservatives, flavoring agents and sweetening agents.

The compounds of the present invention are novel diterpene compounds with antineoplastic activities, and therefore, useful as antineoplastic agents.